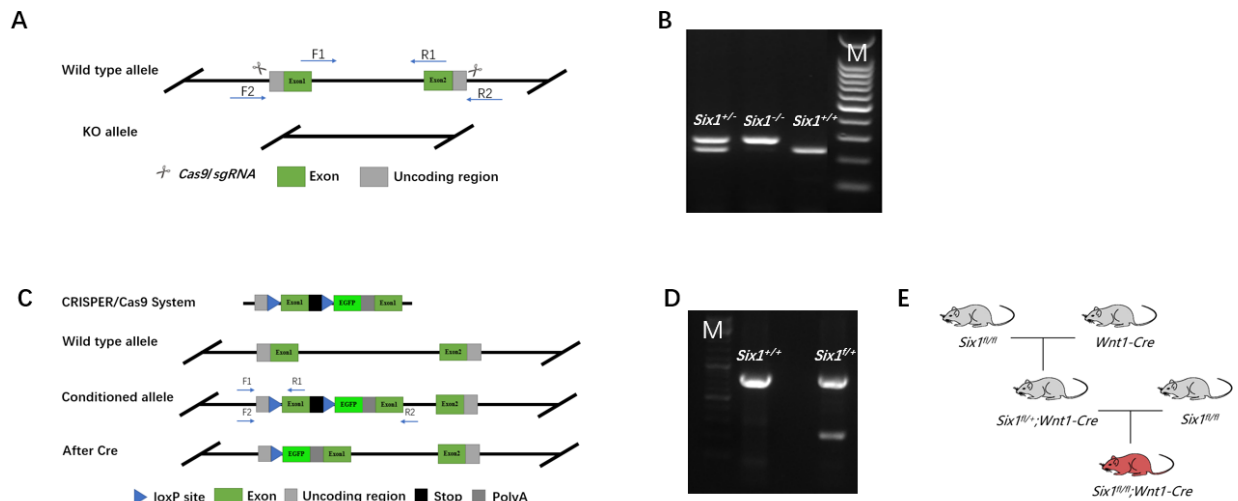
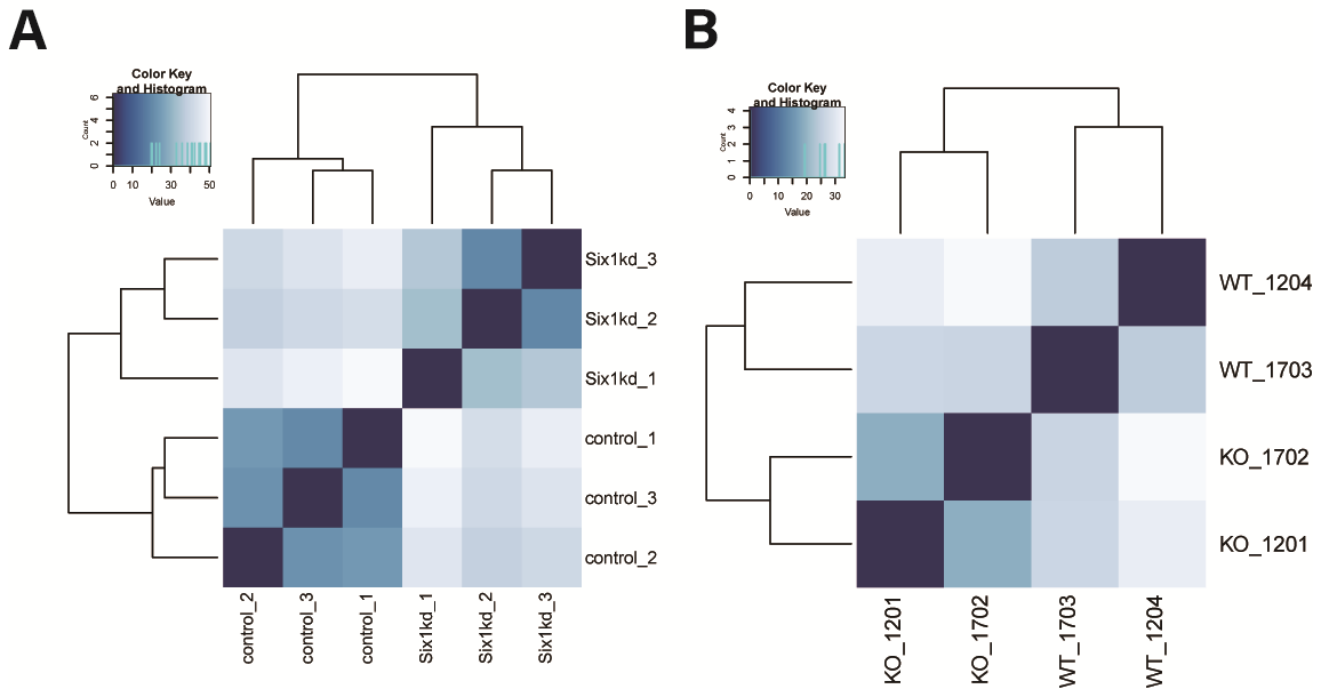


Supplementary Material

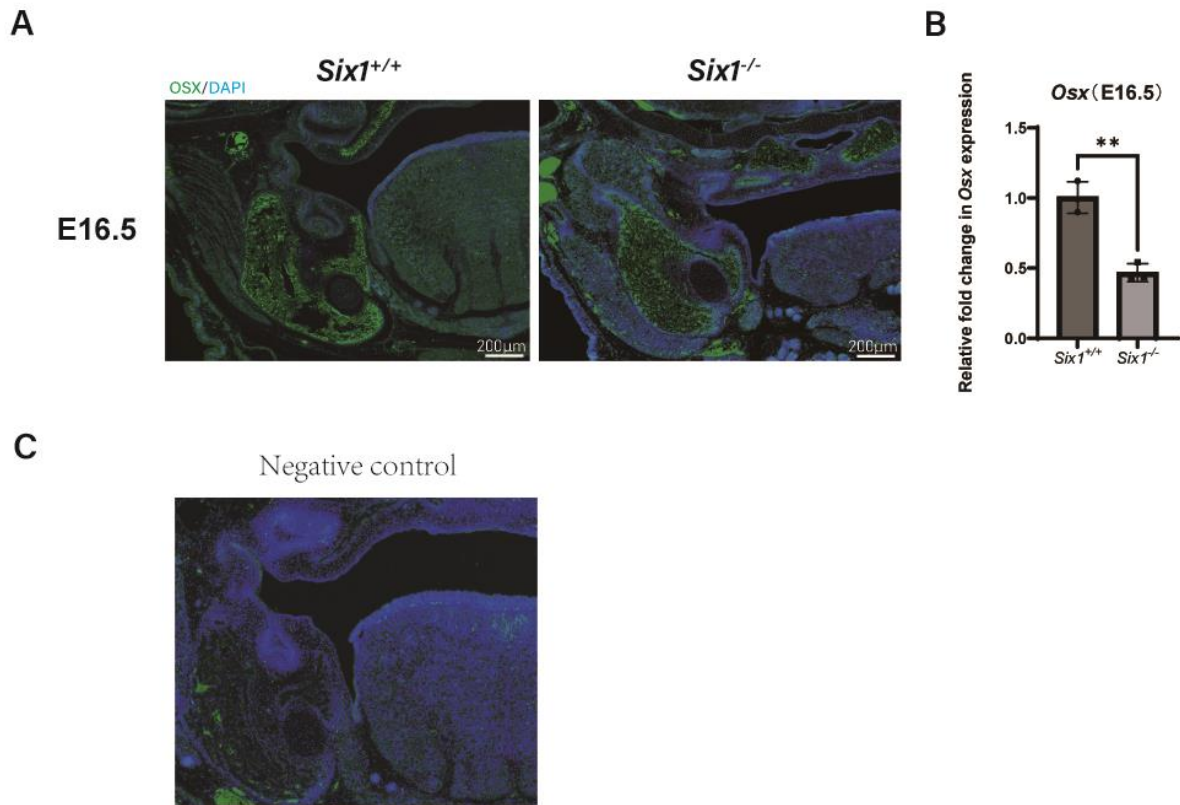
1 Supplementary Figures



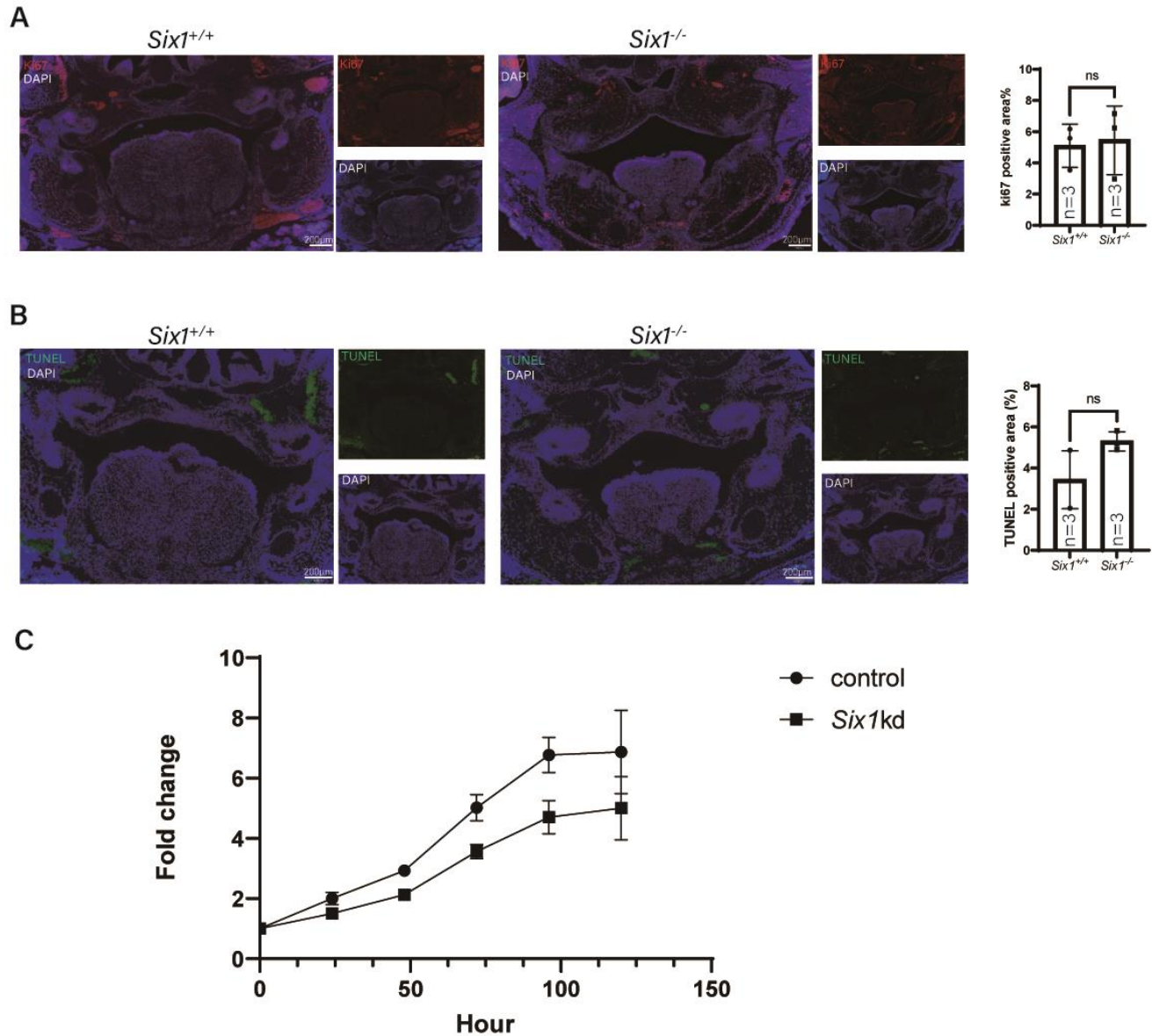
Supplementary Figure 1. (A) Schematic diagram of the generation of *Six1* knockout mice. (B) Genotyping of *Six1* knockout mice. *Six1*^{-/-} mice have a 298bp band, *Six1*^{+/+} mice have a 254bp band, and *Six1*^{+/-} mice have both 298bp and 254bp bands. (C) Schematic diagram of the generation of *Six1* conditional knockout mice. (D) Genotyping of *Six1* conditional knockout mice. *Six1*^{+/+} mice have a 803bp band, and *Six1*^{+/+} mice have both 803bp and 223bp bands. (E) Schematic diagram of the *Six1*^{ff}; *Wnt1*-Cre mouse construction strategy. M, Marker.



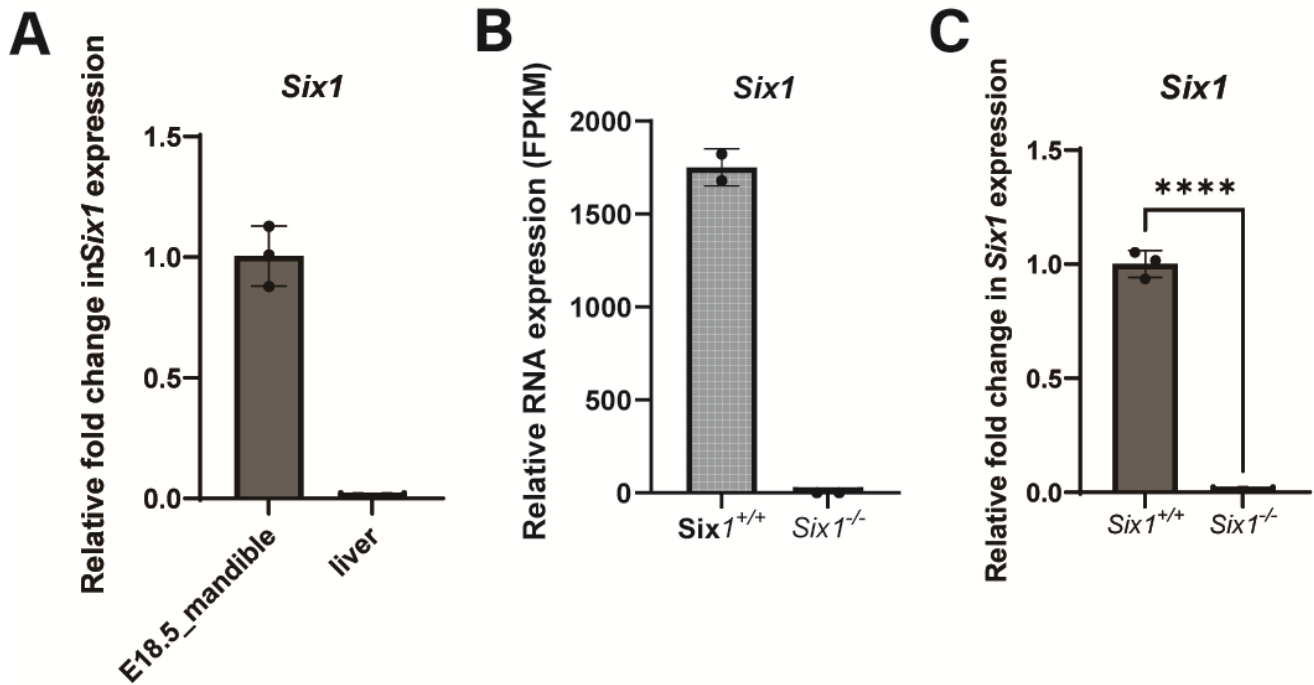
Supplementary Figure 2. (A) Correlation matrix for RNA-sequencing of *Six1* knockdown and control C3H10 T1/2 cells. (B) Correlation matrix for RNA-sequencing of *Six1*^{-/-} and *Six1*^{+/+} embryos at E18.5.



Supplementary Figure 3. (A) Immunofluorescence staining of Osx in mandible of *Six1*^{-/-} and *Six1*^{+/+} embryos at E16.5. (B) RT-qPCR analysis of *Osx* in *Six1*^{-/-} and *Six1*^{+/+} mandibular tissues at E16.5. (C) Negative controls of the immunofluorescence staining.



Supplementary Figure 4. (A) Immunofluorescence staining of Ki67 in mandible of *Six1*^{-/-} and *Six1*^{+/+} embryos at E16.5, and the statistical analysis of Ki-67 positive area. (B) TUNEL assay genotyping of *Six1*^{-/-} and *Six1*^{+/+} embryos at E16.5, and the statistical analysis of TUNEL positive area. (C) Cell proliferation assays were performed for control and *Six1* knockdown C3H10 T1/2 cells at 0, 24, 48, 72, 96, or 120 hours (0–5 days).



Supplementary Figure 5. (A) RT-qPCR analysis of *Six1* in mandible and liver of E18.5 mice. (B) Bar graphs showing the *Six1* expression levels in *Six1*^{-/-} and *Six1*^{+/+} mandibular tissue according to RNA-seq. (C) RT-qPCR analysis of *Six1* in *Six1*^{-/-} and *Six1*^{+/+} mandibular tissue at E18.5.

2 Supplementary tables

TABLE 1 List of primer sets used for genotyping.

Mouse model		Primer (5'-3')
<i>Six1</i> knockout mice	F1:	GCCTCTGCACACCAGAGATAGG
	R1:	GAATACGCACATTCTGGATCGG
	F2:	TTGGGTTTGTTCATGCCCTC
	R2:	CGAAATGAAGCAGAACTCTCCTCC
<i>Six1</i> conditional knockout mice	F1:	ACTTCTCTTCGTCTTCCTTGGCC
	R1:	GGCCCGATAACTTCGTATAGCATAAC
	F2:	ACTTCTCTTCGTCTTCCTTGGCC
	R2:	AAGGGAGCGAAGGGTGAAACTT
<i>Wnt1-cre</i>	F1:	ATTTGCCTGCATTACCGGTCG
	R1:	CAGCATTGCTGTCACTTGGTC

TABLE 2 List of primer sets used for RT-qPCR.

Genes	Forward (5'-3')	Reverse (5'-3')
<i>Alp</i>	CGGGACTGGTACTCGGATAA	ATTCCACGTCGGTTCTGTTC
<i>Osx</i>	CCTTCCCTCACTCATTTCTGG	TGTTGCCTGGACCTGGTGAGAT
<i>Ocn</i>	GAACAGACAAGTCCCACACAG	GAGCTGCTGTGACATCCATAC
<i>Opn</i>	AGCAAGAACTCTTCCAAGCAA	GTGAGATTTCGTCAGATTCATCCG
<i>Etv1</i>	TTAAGTGCAGGCGTCTTCTTC	GGAGGCCATGAAAAGCCAAA
<i>Fgf18</i>	CCTGCACTTGCCTGTGTTTAC	TGCTTCCGACTCACATCATCT
<i>Fgfr2</i>	AATCTCCAACCAGAAGCGTA	CTCCCAATAAGCACTGTCCT
<i>Fat4</i>	TCAGCCAAACCGAGTCA	TCCATTACACAGCCCACA
<i>Runx1</i>	GATGGCACTCTGGTCACCG	GCCGCTCGGAAAAGGACAA
<i>Dlk1</i>	AGTGCGAAACCTGGGTGTC	GCCTCCTTGTTGAAAGTGGTCA
<i>Runx2</i>	GGCCGGGAATGATGAGAACTAC	GGACCGTCCACTGTCACTTT
<i>Bmp4</i>	TGATACCTGAGACCGGGAAG	AGCCGGTAAAGATCCCTCAT
<i>Six1</i>	GAAAGGGAGAACACCGAAAACA	GTGGCCCATATTGCTCTGGA
<i>Gapdh</i>	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

TABLE 3 List of number of input reads and uniquely mapped reads of RNA-sequencing.

Samples	Number of input reads	Uniquely mapped reads
C3H10_control_rep1	36750192	89.66%
C3H10_control_rep2	26716583	91.18%
C3H10_control_rep3	21658547	91.04%
C3H10_Six1kd_rep1	35031085	92.86%
C3H10_Six1kd_rep2	35952420	92.65%
C3H10_Six1kd_rep3	38199370	92.71%
Six1_WT_1204	38041711	87.9%
Six1_WT_1703	36188803	88.66%
Six1_KO_1201	40664817	88.61%
Six1_KO_1702	33502150	89.23%